

MORPHOLOGICAL AND ANATOMICAL FEATURES OF *ALTERNANTHERA*

BETTZICKIANA (REGEL) G. NICHOLSON

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ABSTRACT

Alternanthera bettzickiana (Regel) G. Nicholson is an edible green and has adequate medicinal properties. This present study is aimed at identification of the plant based on macroscopic and microscopic observation. *Alternanthera bettzickiana* is an erect or spreading, bushy colourful ground covering prostrate perennial herb. Stem is an erect or creeping, much branched, with the apical part of the stem is quadrangular, the basal part is cylindrical and hairy at the apex and nodes. Flowers are often axillary sessile spikes or clusters. The anatomy of the root *A. bettzickiana* different thickness was studied. The vascular system of the root exhibits unusual or anomalous type of secondary growth. The vascular system is multi-stranded. There is a horizontal arc of about 10 vascular bundles in petiole of *Alternanthera bettzickiana*. There are two equally developed vascular bundles placed side by side in the midrib region in leaf of *Alternanthera bettzickiana*. The bundles are elliptical in shape and collateral with upper xylem strand and lower phloem strands. The stomata are exclusively diacytic type in *Alternanthera bettzickiana*. Crystals of calcium oxalate druses are fairly common in the ground parenchyma cells of the petiole and mesophyll cells of the leaf of *Alternanthera bettzickiana*. The ovules are broad, elliptical and curved embryo. This study provides referential botanical information for correct identification of the plant.

KEYWORDS: *Alternanthera bettzickiana*, Vascular Bundles, Crystals & Diacytic Stomata

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INTRODUCTION

Amaranthaceae is a cosmopolitan family consisting of 64 genera and about 800 species, mostly abundant in tropical regions of America, Africa and India (Arul Pamila and Karpagam, 2018). *Alternanthera* is the second largest genus in subfamily Gomphrenoideae of Amaranthaceae (Eliasson, 1990 and Townsend, 1993). The genus comprises approximately 80 species which are widespread in the tropical and subtropical regions of New World. *Alternanthera* has 200 species in the tropics and sub-tropics region. The genus *Alternanthera* is represented in India by five species, namely *A. sessilis*, *A. pungens*, *A. tenella*, *A. paronychioides* and *A. bettzickiana* (Sivarajan and Mathew 1984; Naik and Pokle, 1985). *Alternanthera* is enriched with vitamin, flavonoids, glycosides, saponins and other secondary metabolites (Jogendra *et al.*, 2012).

A. bettzickiana (Regel) Nicolson (syn. *Telanthera bettzickiana* Regel (Reddy and Raju, 1997), is an erect herbaceous perennial and an ornamental pot plant in houses and public gardens, often with variegated leaves, growing 20 - 80 cm tall (Standley, 1938). *Alternanthera bettzickiana* is native of South America, commonly known in English as Baptist plant, border plant, red calico plant and cultivated throughout South East Asia and

large cities of China and now distributed in all parts of the world. The plant is sometimes harvested from the wild for its edible leaves. It is widely grown as an ornamental in tropical and subtropical gardens, being valued especially for the colourfully variegated leaves (Standley, 1938). Probably it may be unknown as a truly wild plant and sometimes escapes from cultivation, it is found in open, sunny sites at elevations up to 2,000 meters. Succeeds in full sun to partial shade and prefers an organically rich, consistently moist, well-drained soil. Plants can flower throughout the year (Arthur, 2000). There was no detailed morphological and anatomical description for the plant *A. bettzickiana*, therefore, in the present investigation it was subjected to anatomical studies. The present studies help to identify the plant.

MATERIALS AND METHODS

Collection, Identification, and Authentication of the Plant

The aerial parts of *Alternanthera bettzickiana* (Regel) G. Nicholson was collected from Pechiparai of Kanyakumari District, Tamil Nadu, India. The collected plants were identified in the Department of Botany, Queen Mary's College; Dr. Narashiman, Associate Professor, Madras Christian College, Chennai confirmed the plant and authentication done by Prof. P. Jayaraman, Director, Plant Anatomy Research Centre (PARC) West Tambaram, Chennai. The Reference number of *Alternanthera bettzickiana* (Regel) G. Nicholson is PARK/2016/3464. Herbaria of voucher specimens were prepared and the specimens were lodged in the Queen Mary's College and Plant Anatomy Research Centre.

Preparation of Permanent Microslides

The samples were fixed in FAA (formalin: acetic acid: ethanol in the ratio 5:5: 90 v/v) for 24 h. The specimens were processed as per the procedure of Sass (1940) and sections were made as per Johansen (1940) and staining was done as per O'Brien *et al.* (1964). Photographs of different magnifications were taken with Nikon lab photo 2 microscopic Unit. Descriptive terms of the anatomical features are given as by (Esau, 1964).

RESULTS

Morphological Features

Alternanthera bettzickiana is an erect or spreading, bushy colourful ground covering prostrate perennial herb growing up to 5-45 cm height. The stem and branches are villous when young but soon glabrescent but occasionally reaches a height of 1 m (Figure 1a). A stem is an erect or creeping, much branched, with the apical part of the stem is quadrangular, the basal part is cylindrical and hairy at the apex and nodes. Leaves are opposite, 1-3.5 x 0.5-2 cm, narrowly or more broadly elliptic to oblanceolate or rhomboid-ovate, acute or acuminate at the apex, long-attenuate into an indistinctly demarcated petiole, often purple-suffused and variegated. Leaf blades are green, red, various shades of purple, or sometimes tinged red or yellow, petioles up to 1-2 cm long but sometimes very short or absent. Both the upper and lower surfaces of the leaf blades are pilose and pustular. Twigs are longitudinally ribbed, densely clothed in multicelled, minutely barbed and white hairs. Flowers are often axillary sessile spikes or clusters (Figure 1b). Perianth segments are about 3-5 mm long, clothed in pale hairs (Figure 1c). Androecium staminal filaments fused towards the base and anthers about 1-2 mm long, free filaments, staminodes alternating with the stamens. Staminodes are about 2.5-3 mm long, apices toothed or lobed. Gynoecium ovary about 1 mm long enclosed in the staminal tube (Figure 1d). A style is short and about 0.5 mm long (Figure 1d) Fruits consist of tightly packed bracts interspersed with pale hairs. Fruits are 1 mm long, enclosed in the persistent perianth segments and bracts. Seeds are discoid and testa brown curved embryo.

Anatomical Features of *A. bettzickiana*

Root: Roots of different thickness were studied.

Young (thin) Root: The thin root is 1 mm in diameter. It consists of periderm, cortex and thick vascular cylinder (Figure 2a). The periderm is six-layered and uniformly thick extending all around the root. The periderm cells are rectangular thick walled and suberised. They occur in compact radial rows (Figure 2b). Inner to the periderm occur the cortex which is three-layered with compact, polygonal, and parenchyma cells. Inner to the cortex, a thin continuous cylinder of meristematic cells was found. These cells represent the second ring of vascular cambium which is produced from the cells of the inner tissue of the cortex. The central first formed vascular cylinder consists of the outer zone of secondary phloem and a central dense core of secondary xylem. The secondary phloem includes narrow radial lines of small angular sieve elements. The xylem cylinder comprises of diffuse, solitary vessels of circular, very thick-walled cells. The vessels in the central part of the root are smaller and those towards the periphery are from 20 to 50 μm wide. The xylem fibers are wide, polygonal thick walled and lignified.



Figure 1(a): Habit

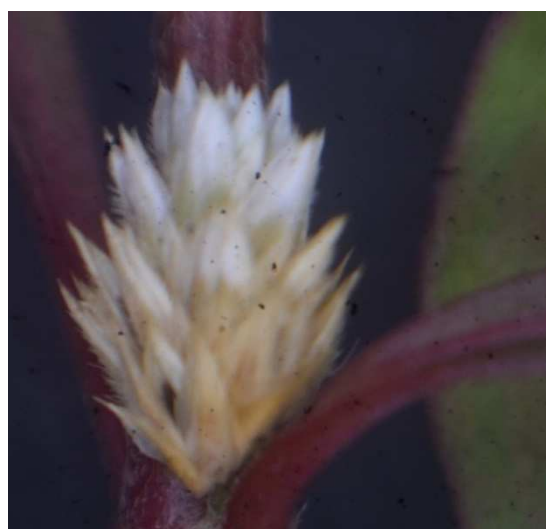


Figure 1(b): Inflorescence



Figure 1(c): Flower



Figure 1(d): Gynoecium

Figure 1: *Alternanthera bettzickiana*

Thick Root: The thick root is 1.2 mm in diameter. It includes thick periderm, narrow cortex, central first formed vascular units followed by second and third formed vascular cylinders (Figure 2c). The root has five layers of superficial periderm, the epidermis is broken and crushed. The periderm cells are rectangular, small and occur in radial rows. The cortex consists of larger polygonal parenchyma cells. The vascular system of the root exhibits the unusual or anomalous type of secondary growth. There is a central, two fan-shaped collateral xylem and phloem; outer to this first formed secondary xylem and phloem, there is an outer ring of secondary xylem and secondary phloem and a third incomplete ring of xylem and phloem.

Thus, the root consists of three successive rings of secondary xylem with secondary phloem. The vascular cylinders possess a short radial chain of narrow or wide, circular thick-walled vessels and narrow thick walled dense fibers. Secondary phloem includes the small cluster of sieve elements and parenchyma cells (Figure 2d).

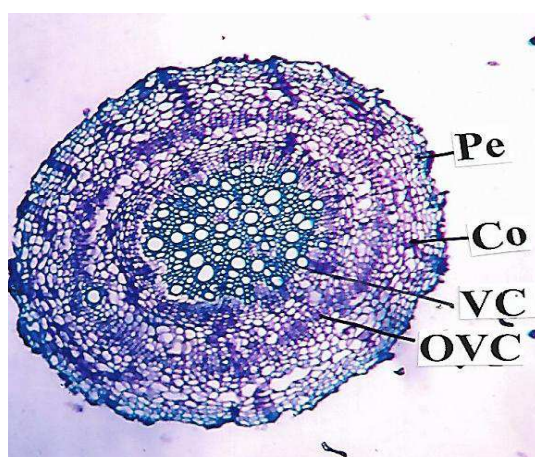


Figure 2(a): T.S. of Thin Root

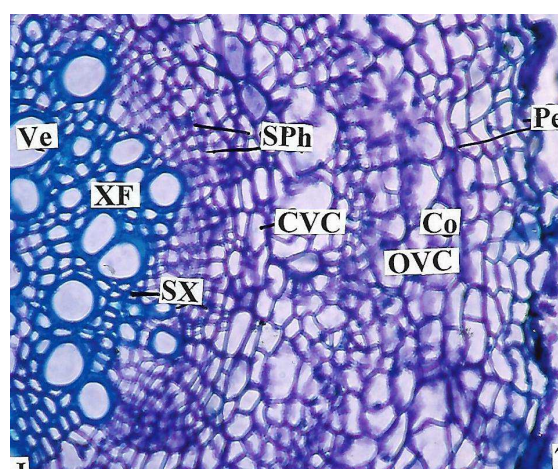


Figure 2(b): Thin Root -Section Enlarged View

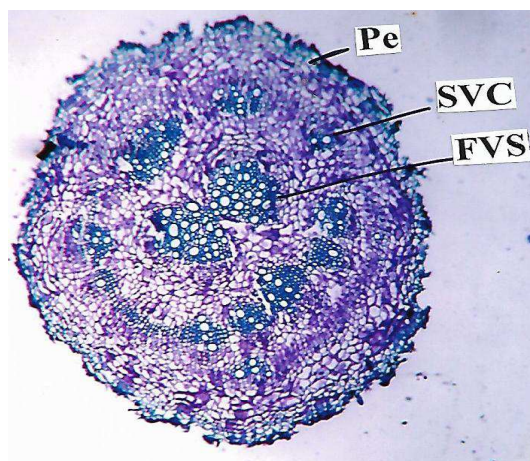


Figure 2(c): T.S. of Thick Root

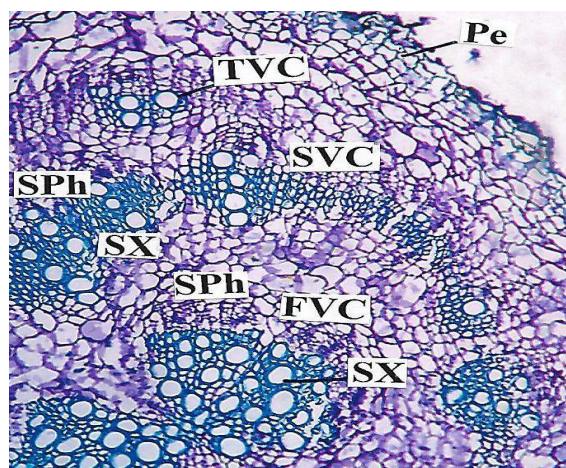


Figure 2(d): Thick Root- Section Enlarged View

Figure 2: *Alternanthera bettzickiana*- Root

(Pe- Periderm, Co- Cortex, VC- Vascular Cylinder, OVC- Outer Vascular Cylinder, SPh -Secondary Phloem, Ve- Vessels, XF - Xylem Fibres, CVC--Central Vascular Cylinder, SX - Secondary Xylem, FVC - First formed Vascular Cylinder, SVC - Second formed vascular Cylinder, TVC-Third formed vascular Cylinder, SX -Secondary Xylem)

Stem: The stem is circular in transectional view and is even and smooth. It is 2 mm thick. The cortical zone is 150 μm thick; the vascular cylinder is 500 μm thick, and pith is 1 mm wide. The epidermis consists of rectangular wide cells with thick cuticle. The outer cortex includes three or four layers of collenchyma and about six layers of wide spindle shapedless compact cylinder in hollow comprising of the outer thick cylinder of secondary phloem and inner cylinder of secondary xylem. The vascular cylinder consists of several radially long triangular segments which are laterally innerconnected by narrow segments of vascular tissues (Figure 3a). The secondary phloem consists of radial, thin lines of small, darkly stained sieve elements and slightly larger parenchyma cells. Thick tangential segments of sclerenchyma cells are located at the distant intervacular region on the peripheral part of the phloem cylinder. The sclerenchyma segments are particularly located on the outer part of the radially elongated triangular segments of the vascular cylinder. Secondary xylem consists of solitary, diffusely distributed vessels. The vessels are circular, wide, thick-walled and lignified. These are both narrow and wide vessels. The diameter of the vessels ranges from 20-40 μm in diameter. The xylem fibers are polygonal in outline. They have very thick lignified secondary walls and wide lumen. The xylem fibers from the ground tissue of the secondary xylem (Figure 3.b.)

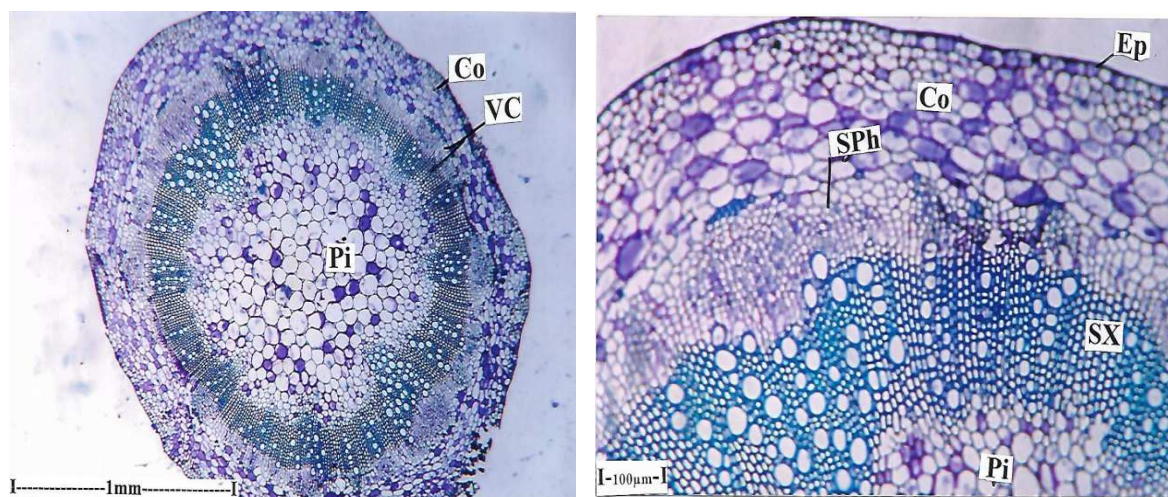


Figure 3(a): T.S. of Stem - Entire View

Figure 3(b): T.S. Stem - A Section Enlarged

Figure 3: *Alternanthera bettzickiana*– Stem

(Co - Cortex, Pi - Pith, VC- Vascular Cylinder, Ep - Epidermis, SPh- Secondary Phloem, SX - Secondary xylem)

Petiole: The petiole is cup-shaped in cross-sectional view with the concavity on the adaxial side (Figure 4a.) The middle part of the petiole is 90 μm thick and 180 μm wide. The wings are 250-350 μm in height and 250 μm thick. The epidermal layers of the petiole have circular fairly wide thin-walled cells. The ground tissue is the homogeneous thin walled parenchymatous cell. The vascular system is multi-stranded. There is a horizontal arc of about 10 vascular bundles. These vascular bundles in the median middle part are larger and the bundles along the wings are smaller. All the bundles are collateral with a cluster of xylem elements on the upper part and phloem on the lower part (Figure 4b). The xylem elements are circular or angular, wide and thick walled. The phloem units consist of small darkly stained sieve elements in three median vascular bundles, the middle one is slightly smaller than the lateral ones (Figure 4b). The wings have wide, vertically standing thick walled semicircular epidermal cells. The ground tissue includes wide, polygonal thick walled and compact parenchyma cells. Small circular vascular bundles are located in a vertical row. Crystals of calcium oxalate druses are fairly common in the ground parenchyma cells of the petiole (Figure 5d). The druses are 30 μm in diameter.

Leaf: In crosssectional view, the leaf is thick with an even surface and less prominent spindle-shaped midrib (Figure 5a). The midrib is 450 μm thick. The midrib has narrow epidermal layers. The epidermal cells are small, circular and thick walled. The epidermal layers on the adaxial and abaxial sides are 20 μm thick. There are two equally developed vascular bundles placed side by side in the midrib region. The bundles are elliptical in shape and collateral with upper xylem strand and lower phloem strands. The vascular bundles are 100 x 160 μm with xylem elements consisting of a wide, compact cluster of narrow angular, thick-walled cells. The phloem elements include a semicircular row of sieve elements and phloem parenchyma cells. The vascular bundles are surrounded by a layer of parenchymatous bundle sheath.

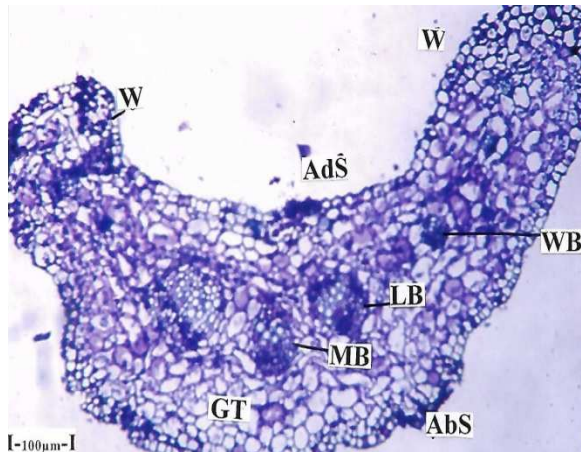


Figure 4(a): T.S. of Petiole

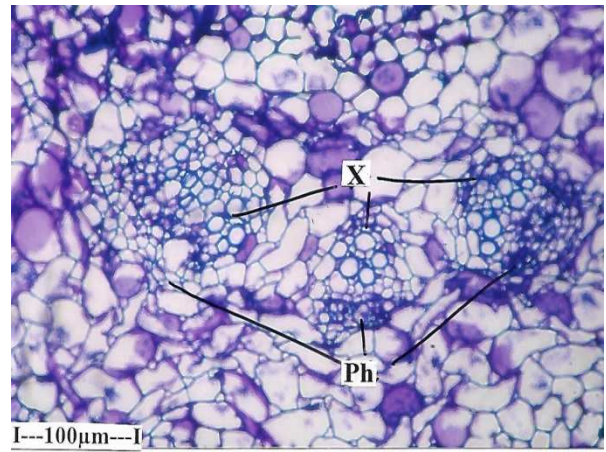


Figure 4(b): Vascular Bundles – Petiole

Figure 4: *Alternanthera bettzickiana*– Petiole

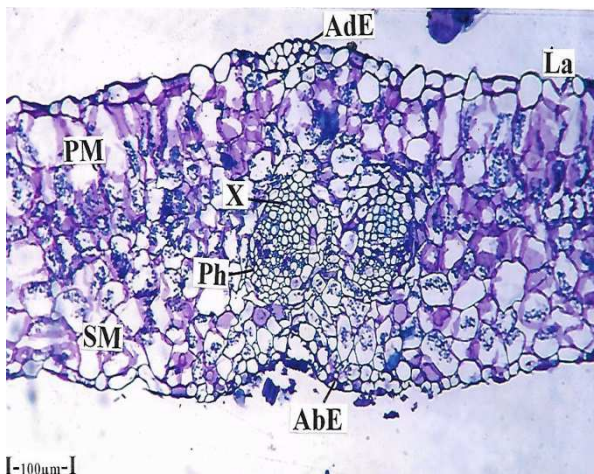


Figure 5(a): T.S. of Leaf

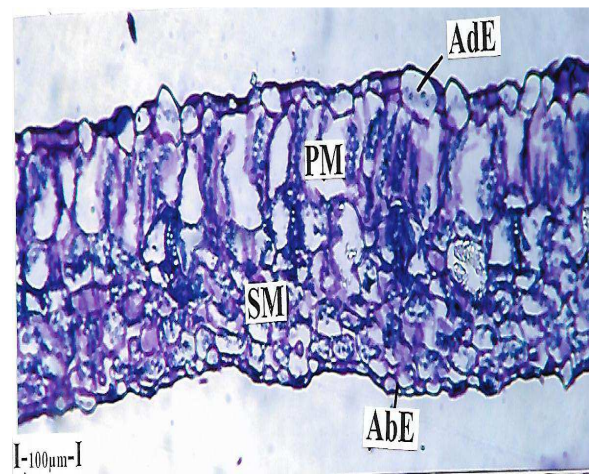


Figure 5(b): T.S. of Leaf Lamina

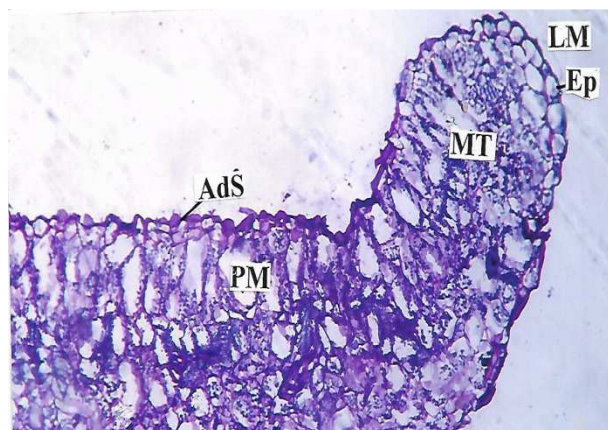


Figure 5(c): T.S. of Marginal Part -Lamina

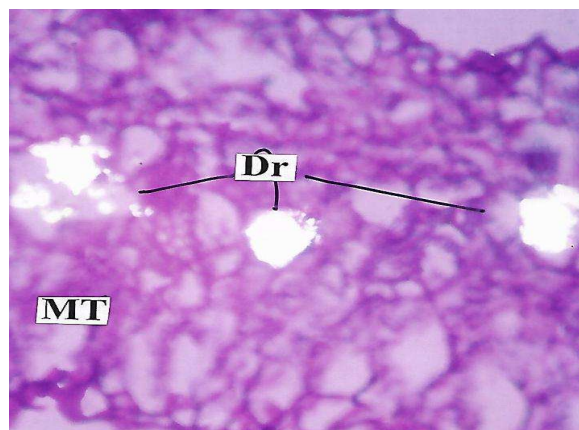


Figure 5(d): T.S. of Leaf Lamina Crystals

Figure 5: Leaf of *Alternanthera bettzickiana*

(W - Wing, Abs - Abaxial side, Ads - Adaxial side, GT - Ground Tissue, LB - Lateral bundle, MB - Median Bundle, Ph- Phloem, WB - Wing Bundle, X - Xylem, Ph- Phloem, AdE- Adaxial Epidermis, La - Lamina, PM- Palisade Mesophyll, SM - Spongy Mesophyll, AbE- Abaxial Epidermis, PM- Palisade Mesophyll, SM - Spongy Mesophyll, MT - Mesophyll tissues, AbE- Abaxial Epidermis, Dr- Druses, MT - Mesophyll tissues, Ep - Epidermis, Id -Idioblast).

Lamina: The lamina is 290 µm thick. It is distinctly dorsiventral with adaxial and abaxial differences. The abaxial epidermal cells are small circular and slightly thick walled. The adaxial epidermis consists of small circular cells as well as prominent conical cells which protrude well above the epidermal level. The mesophyll tissue includes a horizontal layer of palisade cells which are wide and compact. The palisade layer is 100 µm in size. The spongy parenchyma cells are distributed in eight layers. The cells are spherical or lobed loosely interconnected with each other forming wide air-chambers (Figure 5b)

Leaf Margin: The leaf margin is bent upward at right angles to the adaxial surface of the lamina. The leaf margin is 300 µm thick. The epidermal cells along the margin are compact and closely arranged (Figure 5c).

Leaf Venation Pattern: The lamina shows very wide vein islets and fairly abundant vein terminations. The inlets are polygonal in outline; the vein boundaries are straight and within the islets are seen to the simple unbranched and branched once or twice vein terminations. The terminations are thin and undulate (Figure 6a).

Stomata: Stomata are densely distributed in the abaxial epidermis of the leaf. The stomata are the exclusively diacytic type (Figure 6b) The stoma has two subsidiary cells, located at both ends of the guard cells the common walls being at angles to the long axis of the guard cells. The subsidiary cells may be equal or unequal in size. The guard cells are broadly elliptical (Figure 6c & d) The epidermal cells are fairly thick walled. The anticlinal walls of the epidermis are wavy and undulate.

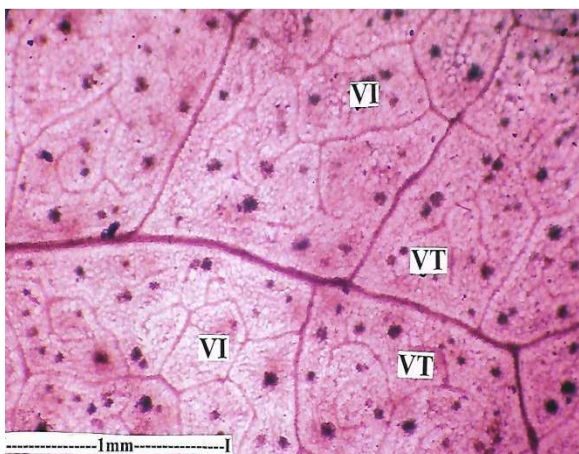


Figure 6(a): Leaf Venation Pattern

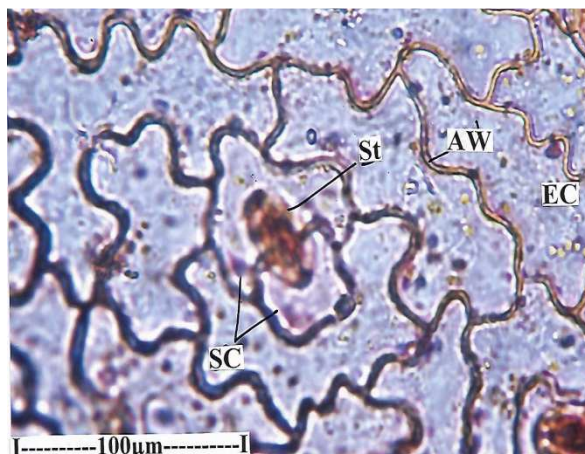


Figure 6(b): Stomata

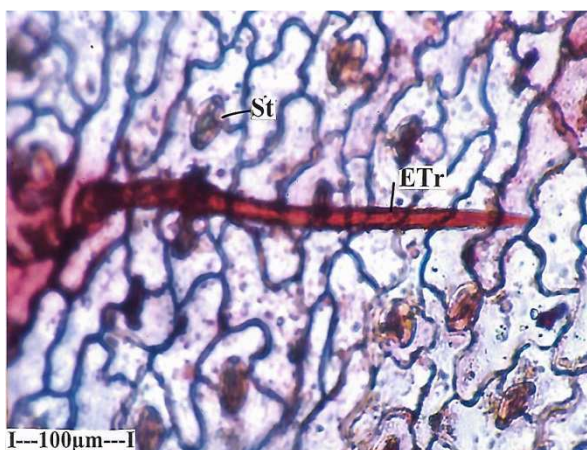


Figure 6(c): Epidermal Trichome

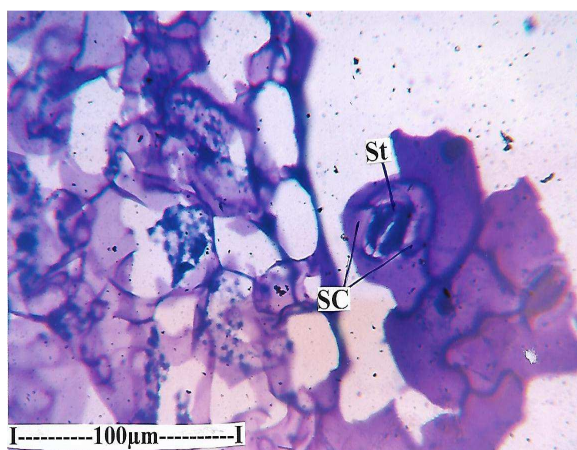


Figure 6(d): Diacytic Stomata

Figure 6: Leaf Venation Pattern and Stomata in *Alternanthera bettzickiana*

(VI - Vein Islet, VT - Vein Terminations, St - Stomata, ETr- Epidermal Trichome, SC- Subsidiary cells, AW -, EC -Epidermal cells)



Figure 7(a): Clusters of Trichome

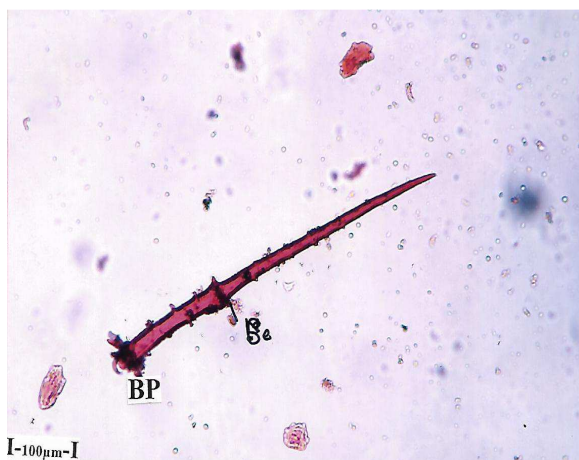


Figure 7(b): Trichome Single

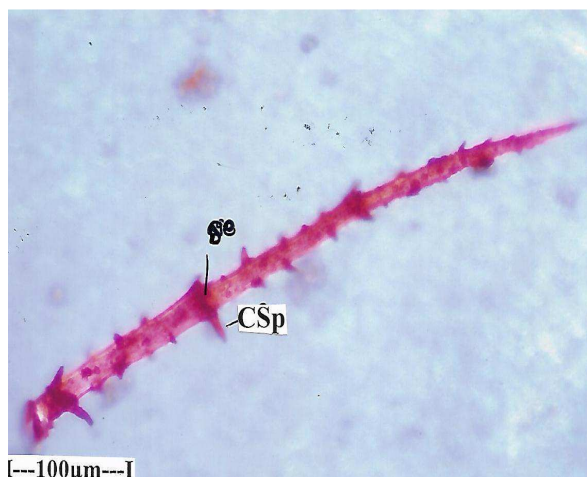


Figure 7(c): Trichome Cuticular Spines

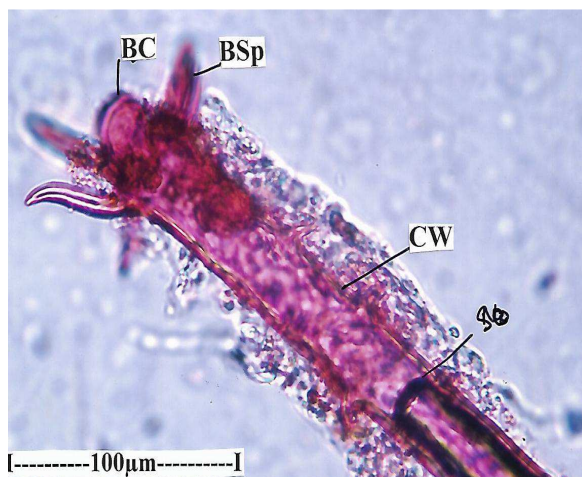


Figure 7(d): Basal Anchoring Lateral Outgrowths

Figure 7: Trichomes in *Alternanthera bettzickiana*

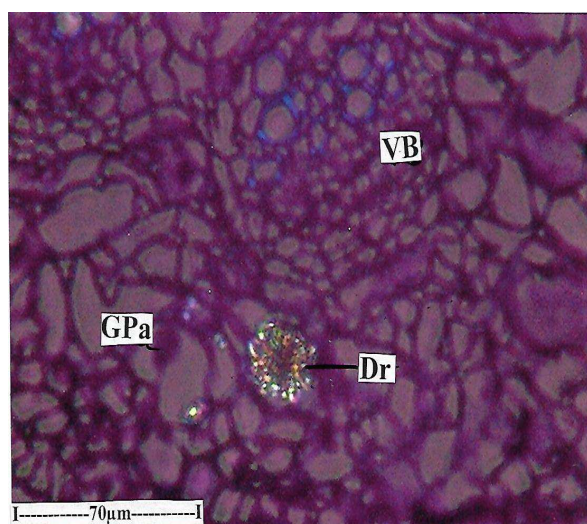


Figure 8(a): Calcium Oxalate Druses

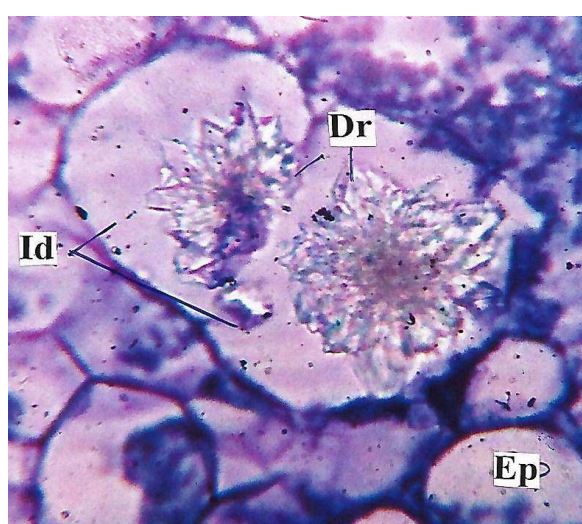


Figure 8(b): Idioblasts of Mesophyll Tissue

Figure 8: Crystals in *A. bettzickiana*

(Tr- Trichome, Se- Septum, BSp - Basal spiny outgrowths, BC- Basal Cell, CSp -Cuticular spines, CW- Cell Wall, VB- Vascular Bundle, GPa - Ground Parenchyma, Dr - Druses, Ep - Epidermis, Id - Idioblast)

Epidermal Trichomes: Non-glandular multicellular, uniseriate unbranched epidermal trichomes are abundant on the leaf surface of the abaxial side (Figure 7.a & b.) The trichomes are long, thick and pointed at the tip. The trichomes are embedded in the epidermis by a short cylindrical basal cell and three or more short thick walled epidermal cells all around the basal cells anchoring the trichome on the leaf (Figure 7c.) Along the entire length of trichomes, there are short, sharp thick cuticular spines (Figure 7d). The trichomes are 600-850 μm long and 30 mm thick at the base.

Crystal Distribution: Calcium oxalate druses are common in the mesophyll cells of the leaf (Figure 5d) The crystals are large measuring 50 μm in diameter. The druses are located individually in the cells. The cells bearing the druses are slightly enlarged (Figure 8a). Fairly large, spherical bodies with the dense conical spiny surface are common in the mesophyll tissue of the leaf (Figure 5d). These crystals are called druses or sphaerocrystals. The druses are located in

the dilated, specialized parenchyma cells called idioblasts. Usually, only one druse occurs in an idioblast (Figure 8b.) The calcium oxalate crystals are 30 μm in diameter.

Floral Anatomy of *Alternanthera bettzickiana*

The flowers are small and are grouped into axillary clusters. During the sectioning more than one flower are cut into transverse plane. (Figure 9a). There are five petals which are pale, white and similar in external features. Hence the petals are called tepals out of five tepals two are larger and the other three are smaller. The tepals are imbricate in aestivation with two larger tepals are completely out and the remaining tepals have inner and outer margins persistent.

Tepals

Outer Tepal

The outer tepal has a single midrib which is thick with flat inner side and convex outer side. The lateral region becomes gradually tapering into a single layer of cells. The midrib region is 50 μm thick and the wing region is 20 μm thick. The outer epidermis of the outer tepal consists of highly thick walled semicircular cells with a reduced lumen (Figure 9c). The epidermis also consists of thick-walled hemispherical cells similar to the outer epidermis. There are two or three layers of highly thick walled lignified sclerenchyma cells located in the adaxial epidermis. In between the outer and inner epidermal layers occur four layers of polygonal, thick-walled compact mesophyll tissue. In the midrib region occur two small clusters of xylem elements which are small, angular and thick walled. Two or three phloem elements are associated with the xylem elements (Figure 9a).

Inner Tepal

The inner tepal is thick in the midrib region and is tapering in the ends. The tepal gradually tapers into a single layer of whiplike structure (Figure 9a). The inner and outer epidermal layers are highly thickly walled and lignified with a narrow lumen. The inner concave region has two or three additional thick walled lignified cells. The midrib is 50 μm thick; the submarginal region is 20 μm thick, the extreme wing is 5 μm thick and it consists of a single layer of circular cells.

Anther

The anthers are dithecal and two-celled (Figure 9b). The stamen has a short thick filament and cylindrical anther. The anther is 400 μm long and 180 μm wide. The pollen grains are spherical, measuring up to 40-50 μm in diameter. The exine of the pollen has minute reticulate thickenings (Figure 9c.)

Ovary

The gynoecium is subsessile with a short thick stalk. The gynoecium is broadly conical with the dilated ovary, thick short style and hairy stigma (Figure 1d). In the cross-sectional view, the ovary is broadly elliptical with a thick parenchymatous wall. The ovule is attached to the ovary wall by short, less distinct funiculus. The ovules are broad, elliptical and curved embryo (Figure 9d).

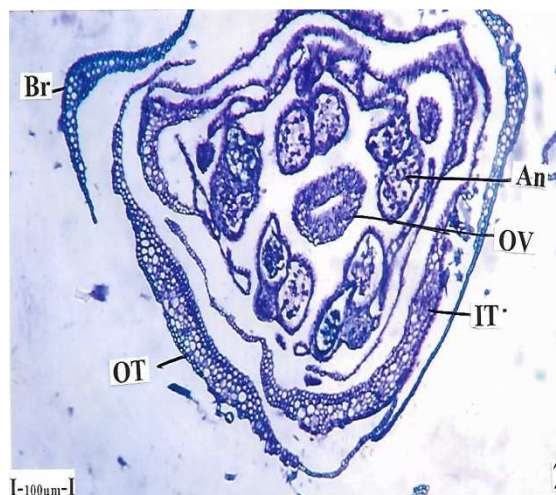


Figure 9(a): T.S. of Flower

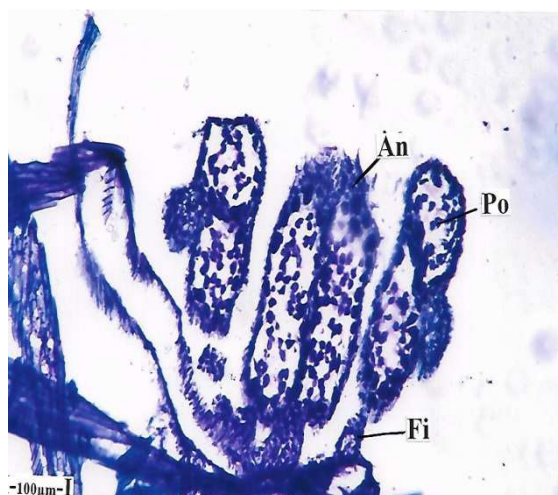


Figure 9(b): L.S. of Anther



Figure 9(c): Pollen

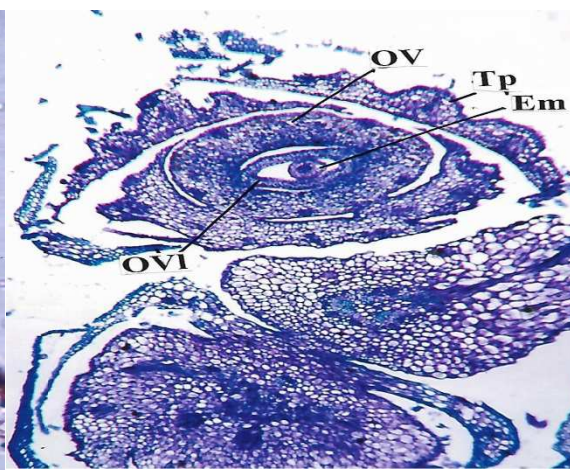


Figure 9(d): T.S. of Ovary

Figure 9: Floral Anatomy - *Alternanthera bettzickiana*

(Br - Bract, OT - Outer Tepal, An -Anther, OV- Ovary, IT - Inner Tepal, An - Anther, Po - Pollen, Fi - Filament, Po- Pollen, Em-Embryo, OV- Ovary, Ove- Ovule, Tp-Tepal).

DISCUSSION

Alternanthera bettzickiana is an erect, branched, perennial herbs, stem pilose and hairy along two vertical lines. Leaves elliptic or oblanceolate, acute or acuminate at the apex, base attenuate. Heads globose, sessile, axillary or terminal. Bracts 2-3.5 mm long, lanceolate or ovate-lanceolate to ovate, acute, mucronate, hairy on back. Bracteoles ovate-lanceolate, acute, mucronate, glabrous or hairy on the back. Tepals 5, unequal; outer three, ovate lanceolate, acute, mucronate; inner 2 short, lanceolate. Stamens 5, pseudostaminodes apically irregularly toothed. Ovary obovoid; style short, stigma capitate (Singh *et al.*, 2008). There are no previous anatomical studies on *A. bettzickiana*. Dutta (2015) described vascular bundle as collateral and closed bundle sheath located in mesophyll tissues of *A. philoxeroides*. In the present study, the vascular system is multi-stranded with a horizontal arc of about 10 vascular bundles, median middle ones are larger and the peripheral bundles are smaller. The stomata are exclusively diacytic type in *A. bettzickiana* whereas stomata are diacytic type in *A. philoxeroides* (Dutta, 2015). Concerning the stem organization in secondary growth, the Amaranthaceae family has an unusual development characterized by a sequence of concentric extra cambium, formed

outside the original cambium (Anitha and Kanimozhi 2012). The cork was 4-5 layers of parenchymatous rectangular cells; the cortex is four to five layers loosely arranged parenchymatous cells, indistinct endodermis and pericycle; narrow phloem and radially arranged tracheary elements (Gupta *et al.*, 2013). The vascular system of the root exhibits the unusual or anomalous type of secondary growth in the thick root of *A. bettzickiana*. In *A. sessilis*, the adaxial surface shows polygonal epidermal cells and abaxial cells are sinuous with rosette aggregates of calcium oxalate crystals (Gupta *et al.*, 2017). Calcium oxalate crystals are present in the stem and leaves of *A. sessilis*. Calcium oxalate druses are common in the mesophyll cells of the leaf of *A. bettzickiana*.

CONCLUSION

The above characters observed in the study clearly specify the plant as *Alternanthera bettzickiana*. This study forms a base for the further pharmacognostical and pharmacological investigation of this plant.

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